allowed to bind the species to be determined (or another species that is indicative of the species to be determined), and a second signaling entity allowed to bind the first entity, a third signaling entity may be allowed to bind the second entity, etc., and some or all of these entities, may be determinable, e.g., fluorescent.

[0074] A non-limiting example of the use of a signaling entity is shown with reference to FIG. **2**. In this figure, a fluidic droplet **20** contains a signaling entity **25** and a cell **22**. Signaling entity **25** comprises a microparticle **26** and a plurality of agents **28**, which may be, for example, a protein, a polypeptide, a peptide, a nucleic acid, an antibody, an enzyme, etc. In some cases, more than one type of agent may be used. Cell **22** may produce a species **29** which is a binding partner to some or all of agents **28**. The signaling entities can then be used to determine production of species **29** by cell **22**. For instance, if species **29** is expressed on the cell surface, the signaling entities will become associated with the cell, e.g., localized within portions of fluidic droplet **20**. If species **29** is released from inside the cell (including by secretion or by lysis of the cell), species **29** may become associated with the signaling entities. As another example, as is shown in FIG. **2**, a second signaling entity **30** may be used that is able to bind to species **29**. If species **29** is present, second signaling entity **30** may become associated with signaling entity **25** as it binds to species **29**; conversely, if species **29** is not present, there may be little or no association of signaling entity **25** and second signaling entity **30**. Second signaling entity **30** may be present when droplet **20** is first formed; or, as shown in FIG. **2**, second signaling entity **30** can be introduced into droplet **20** by the coalescence of droplet **20** with another fluidic droplet containing signaling entity **30**. Non-limiting examples of droplet coalescence are discussed in U.S. patent application Ser. No. 11/246,911, filed Oct. 7, 2005, entitled "Formation and Control of Fluidic Species," published as U.S. Patent Application Publication No. 2006/0163385 on Jul. 27, 2006; or U.S. patent application Ser. No. 11/360,845, filed Feb. 23, 2006, entitled "Electronic Control of Fluidic Species," published as U.S. Patent Application Publication No. 2007/000342 on Jan. 4, 2007, each incorporated herein by reference.

[0075] In some cases, as is shown in FIG. **2**, the droplets may be analyzed to determine species **29**, for example, using a sensor as is discussed below. For instance, if species **29** is present in a droplet, the droplet may be sent to a first location **31** (e.g., for further processing, collection as is shown in FIG. **2**, or the like); if species **29** is absent (or is present, but in an undesirable amount, concentration, configuration, etc.), the droplet may be sent to a second location **32** (e.g., for further processing, waste, or the like). As shown in FIG. **2**, electrodes **35** are used to control movement of the droplets towards first location **31** or second location **32**, e.g., as is discussed in U.S. patent application Ser. No. 11/360,845, filed Feb. 23, 2006, entitled "Electronic Control of Fluidic Species," published as U.S. Patent Application Publication No. 2007/000342 on Jan. 4, 2007, incorporated herein by reference. However, in other embodiments, other systems, e.g., fluidic control, may be used to control the sorting of the droplets. The sensor may include, for example, light (such as a laser) **33** that is directed to the droplets, and the interaction of the light with the droplets may be used to sort or screen the droplets. In some cases, selected droplets can be captured for further analysis, e.g., as is shown in FIG. **2** with array **38**. In some embodiments, sorting may be performed as part of a fluorescent-activated cell sorting (FACS) system.

[0076] As described herein, one or more signaling entities may be added into the droplets to determine amounts of specific species in the droplet, e.g., molecules produced by a cell (e.g., antibodies) within the droplet, and/or measurement of those species' affinity for binding to a target (e.g., a protein). The signaling entities may also be used, in some cases, to measure those species' relative specificity for binding to one target compared to a second or a third target, for example. Each particular choice of signaling entity may allow, in some embodiments a particular method to implement a screen or selection.

[0077] A non-limiting example of a class of signaling entities includes a known quantity of a fluorophore-labeled antigen or "labeled target antigen" (e.g., a FITC labeled phosphopeptide). The labeled target antigen may be contained in a droplet along with a bead coated with a known number of anti-human heavy chain antibodies. In one embodiment, the droplet contains a human B cell that secretes antibodies that bind to both the labeled target antigen and the anti-human heavy chain antibodies on the bead. By measuring the fraction of total fluorophore on the bead, one can measure the affinity of the cell-produced antibody for the target antigen. If a large number of secreted antibodies are bound to the bead, a large fraction of the labeled antigen is on the bead, which shows the secreted antibody has a high affinity for that antigen.

[0078] As yet another example, one can add to the droplet a known quantity of an unlabeled related antigen, a "competitor" (e.g., the same labeled target antigen as above but without phosphorylation), which competes with binding to the secreted antibody. The amount of the fluorophore-labeled antigen bound to the bead is reduced if the secreted antibody has significant relative affinity for the competitor.

[0079] As still another example, the competitor may be labeled with a third color fluorophore (or second if the tracking agent is not used) so that the ratio of target antigen color to competitor color on the bead is a measure of their relative affinity, and the sum of the two colors is a measure of the amount of secreted antibody on the bead.

[0080] The example of the signaling entities above involves, in some cases, binding of an antibody to the bead, for example, through a general anti-heavy chain linker (although other linkers are also possible, as is known to those of ordinary skill in the art). In another embodiment, the target antigen is presented on the surface of the bead, e.g., by covalently linking it to the bead. In this example, the signaling entity may comprise an anti-human heavy chain antibody with a fluorophore label. When one measures that color on the bead, it is a measurement of the amount of cell-secreted antibody that is bound to the target antigen on the bead surface. This example also can be extended to involve the use of a related antigen as a competitor; in this case, the competitor reduces the amount of cell-secreted antibody bound to the bead in direct proportion to the relative affinity of the competitor and the target antigen to the cell-produced antibody.

[0081] Many of the methods and articles described herein may involve the use of more than one signaling entity, e.g., two signaling entities that have different colors for two-color detection. For example, in a fluorescence-concentration assay used to select cells which secrete a desired antibody, the signal generated from a large amount of medium-affinity